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Comparative pharmacokinetics of perfluorohexanesulfonate (PFHxS) in rats, mice, and monkeys

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ABSTRACT

Perfluorohexanesulfonate (PFHxS) has been found in biological samples from wildlife and humans. The human geometric mean serum PFHxS elimination half-life has been estimated to be 2665 days. A series of studies was undertaken to establish pharmacokinetic parameters for PFHxS in rats, mice, and monkeys after single administration with pharmacokinetic parameters determined by WinNonlin® software. Rats and mice appeared to be more effective at eliminating PFHxS than monkeys. With the exception of female rats, which had serum PFHxS elimination half-life of approximately 2 days, the serum elimination half-lives in the rodent species and monkeys approximated 1 month and 4 months, respectively, when followed over extended time periods (10–24 weeks). Collectively, these studies provide valuable insight for human health risk assessment regarding the potential for accumulation of PFHxS in humans.

1. Introduction

Perfluorohexanesulfonate (PFHxS) is one of a number of functionalized, perfluorinated compounds that have been produced for over half a century for use in specialized applications [1] as well as becoming the subject of increasing investigation with regard to environmental and health-related properties [2]. The unique properties of this and other perfluorinated surfactants, such as high surface activity, exceptional stability to degradation, density, solubility characteristics, and low intermolecular interactions, have been exploited in numerous industrial and consumer applications [3]. However, these same properties also create challenges for managing these materials in the environment.

Due to its stability and use in product applications such as fire-fighting foam and carpet, fabric, and upholstery stain protectors, it may not be surprising that PFHxS initially was found in pooled serum from the United States general population [4]. The geometric mean serum PFHxS elimination half-life in 26 retired production workers was estimated to be approximately 7.3 years (95% CI = 5.8–9.2), suggesting poor elimination of PFHxS in humans [5]. Exceptional stability to environmental and metabolic degradation together with poor elimination from the body in the case of several perfluorinated surfactants [5], including PFHxS, create a potential for accumulation and biomagnification. Accordingly, 3M Company, the major manufacturer of PFHxS in the past, phased out the production of these compounds and associated products between 2000 and 2002.

Numerous biomonitoring studies have found PFHxS widely distributed at low ng/mL concentrations in individual samples from the general population [6–12]. The National Health and Nutrition Examination Survey (NHANES), a representative sample of the United States general population aged 12 and older, reported a geometric mean serum PFHxS concentration of 1.96 ng/mL (95% CI = 1.76–2.17) for the 2007/2008 survey period [11]. This geometric mean was similar to the 1999/2000 NHANES survey-period geometric mean of 2.13 ng/mL (95% CI = 1.91–2.38) [11], suggesting that serum PFHxS concentrations in the general population

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from 1999/2000 through 2007/2008 are not decreasing or are doing so at a much lower rate than was observed in the same study for serum concentrations of the eight-carbon congener, perfluorooctanesulfonate (PFOS), which showed a 57% decrease in serum geometric mean concentration, consistent with its estimated mean serum elimination half-life of 4.8 years (95% CI = 4.0–5.8 years) [5]. However, the NHANES data did show a decline from the 1999/2000 geometric mean of 2.14 to 1.67 ng/mL in the 2005/2006 sampling period, a reduction of 22%, but the geometric mean increased to 1.96 in the 2007/2008 survey. Olsen et al. [7] reported an approximately 30% (0.4 ng/mL) decline in geometric mean serum PFHxS among American Red Cross blood donors from six regional blood donation centers between 1999/2000 and 2006, consistent with the NHANES data through 2006. PFHxS has been measured in cord blood [13–15] and newborn blood spots [10]. Evaluations of dried blood spot samples obtained from the Newborn Screening Program in New York State by Spliethoff et al. [10] demonstrated that the mean whole-blood concentration of PFHxS among 10 sample pools was 2.4 ng/mL in 2000, declining to approximately 1.3 ng/mL in 2007, an approximate 46% reduction in the mean value. Trend analysis produced a statistically significant decreasing trend with a halving time of 8.2 years, consistent with the serum PFHxS elimination half-life reported by Olsen et al. [5]. In a longitudinal study of serum PFHxS in men, mothers, and children from three cities in Germany [16], geometric men serum PFHxS was found to decrease in all three cities and groups in the 2-year time period spanning 2006 and 2008 with percent reductions in the geometric mean ranging from 14.3% to 41.4%.

Although the distribution of PFHxS has typically been focused on blood-based media, human milk also has been studied [17,18]. In a temporal trend study with pooled human milk samples from Stockholm, Sweden, Sundström et al. [17] reported an apparent but non-statistically significant decreasing trend of 6.1% per year in the pooled sample concentrations from 2001 through 2008, associated with a halving time of 11 years.

These biomonitoring studies may provide evidence in support of a low elimination rate for PFHxS in humans, or, they may suggest continued low-level environmental exposure, or perhaps both of these plausible explanations. Active environmental exposures to PFHxS may still exist, because PFHxS has been frequently detected in house dusts in samples collected between 2000 and 2008 [19–22]. The dust containing PFHxS potentially could originate from carpet and upholstery that were previously treated with PFHxS-containing surface protectant products. Based on a study from The Netherlands, dietary sources may also contribute to PFHxS exposure [23].

A study of children serum concentrations of PFHxS and other fluorochemicals may provide some insight into the potential role of household exposures to PFHxS. Olsen et al. [12] surveyed PFHxS serum concentrations in 598 children aged 2–12 who participated in a national multi-center study of Streptococcal group A infection between January 1994 and March 1995. In that time period, the geometric mean of the childrens' serum PFHxS was 4.5 ng/mL (95% CI = 4.1–5.1), with boys having a somewhat higher geometric mean than girls (5.3 ng/mL versus 3.4 ng/mL, respectively). The distribution of serum PFHxS in the children appeared to be bimodal, with 11% having serum PFHxS greater than 30 ng/mL, 64% of those values being for boys. Because PFHxS residues may have been present in carpet and upholstery treated for stain resistance, the authors speculated that exposure patterns unique to children, such as playing on treated surfaces, may have accounted for the apparent bimodal distribution. Higher PFHxS concentrations were reported by Kato et al. in analysis of pooled children samples from NHANES [24]. Duplicate pooled samples from the 2001/2002 NHANES were used for each of two age categories (3–5 years and 6–11 years) divided by sex into three ethnic pools (non-hispanic whites, Mexican Americans, and non-hispanic blacks). Although the pooled nature of the samples limits the ability to make inferences, the mean pooled PFHxS serum concentrations in the children were generally higher than means for pooled 2001/2002 NHANES adolescents and adults. This observation of higher values in children than adolescents and adults was also made by both Olsen et al. [12] and Kato et al. [24] for a component of carpet and fabric protection chemistry formerly manufactured before the 3 M phaseout, *N*-methyl-*N*-(2-ethoxy)-perfluorooctanesulfonamide. These observations suggest a unique exposure pattern to PFHxS for children, and exposure to carpeted or upholstered surfaces treated with formulations containing PFHxS is a potential contributing factor.

Several cross-sectional epidemiological studies have evaluated associations of serum PFHxS with various health outcomes. No association of serum PFHxS concentrations have been found with: atopic dermatitis and serum IgE in Taiwanese based on cord blood concentrations of PFHxS taken in 2004 and evaluation of serum IgE and parent-reported atopic dermatitis at 2 years of age in 2006 [15]; thyroid hormones in New York anglers [25] with samples taken between 1995 and 1997 and in pregnant women from Edmonton, Alberta, Canada in a case–control study from 2005 to 2006 [26]; with maternal serum PFHxS and fetal weight and length of gestation in births from Alberta, Canada between 2005 and 2006 [27]. Stein and Savitz [28] reported a positive association of serum PFHxS concentrations in children aged 5–18 with parent- or self-reported ADHD with medication based on serum samples taken in the 2005/2006 time period (odds ratio 1.59, 95% CI = 1.21–2.08). Nelson et al. [29] found a negative association of total and non-HDL cholesterol and no associations with body size and insulin resistance with serum PFHxS based on cross-sectional analysis of NHANES data from the 2003/2004 survey period. This contrasted with positive associations with serum non-HDL cholesterol that were found for serum PFOS and perfluorooctanoate (PFOA). These studies are cross-sectional in nature and none have identified clear, causal associations of serum PFHxS with health outcomes in humans. Furthermore, none of these investigators has followed up with methodologically superior epidemiological study designs.

In contrast to its eight-carbon congener, PFOS, which has been extensively studied for potential health effects [2], there are only a few published studies related to the potential toxicological properties of PFHxS. In a study designed to investigate potential reproductive, developmental, systemic toxicological, and neurological responses, Butenhoff et al. [30] exposed male and female rats to potassium PFHxS by oral gavage at dose levels of 0, 0.3, 1, 3, and 10 mg/kg-d for 2 weeks prior to mating and during mating, gestation, and lactation (postnatal day 22) for parental females as well as during mating and through study day 42 for males (6 weeks). The F1 offspring were sacrificed on postnatal day 22 at the end of weaning. There were no treatment-related effects in maternal rats or their offspring. In males, notable effects included increased liver-to-body weight and liver-to-brain weight ratios, centrilobular hepatocellular hypertrophy, hyperplasia of thyroid follicular cells, and decreased hematocrit. The mean serum PFHxS concentrations in males across the PFHxS dose levels ranged from 44 μg/mL at 0.3 mg/kg to 201 μg/mL at 10 mg/kg. In pooled pup serum from post natal day 22, serum PFHxS concentrations ranged from 9 μg/mL at 0.3 mg/kg to 94 μg/mL at 10 mg/kg. At the end of gestation, maternal serum PFHxS ranged from 3 μg/mL at 0.3 mg/kg to 60 μg/mL at 10 mg/kg. These serum concentrations were at least three orders of magnitude higher than the geometric mean values reported for human general populations. PFHxS has been demonstrated as an agonist for both the human and the mouse forms of the xenosensor nuclear receptor NR1C1 (peroxisome proliferator activated receptor alpha, or PPARα) [31]. Therefore, the hepatic hypertrophic effects observed in the study by Butenhoff et al. likely resulted from activation of

PPARα. Expression of PPARα was observed in both human and rat primary hepatocytes in culture with 25 μM potassium PFHxS for 24 h based on qRT-PCR for Cyp4A1 (rat) and Cyp4A11 (human) mRNA [32], while mRNA expression for Acox was not increased in the same human hepatocyte populations, as it was in the rat hepatocytes. Consistent with its eight-carbon congener, PFOS, PFHxS reduced serum total cholesterol and triglycerides in male APOE*3.Leiden.CETP mice fed approximately 6 mg/kg-d potassium PFHxS in their diet via enhanced lipoprotein-lipase-mediated clearance of triglycerides (TG) associated with very-low-density lipoproteins, as well as decreased production of VLDL-TG and VLDL-apolipoprotein B [33]. Also, decreased apolipoprotein A1 synthesis led to decreased HDL production, resulting in decreased serum HDL.

The presence of PFHxS at low ng/mL concentrations in human general population serum, its low elimination rate in humans, and the possible continuing exposure from sources such as house dust and diet warrants a need to understand potential health risk associated with exposure and the potential association of serum concentrations with effects. As limited pharmacokinetic and toxicological data were available for PFHxS [30], the purpose of the studies reported herein was to establish pharmacokinetic parameters for PFHxS in monkeys, rats, and mice.

2. Materials and methods

2.1. Materials

The potassium salt of PFHxS (K^+PFHxS, >99% pure, 96.1% linear isomer, 1% internal monomethyl branch, 2.8% isopropyl branch) was supplied by 3M Company (St. Paul, MN). Administered doses are for K^+PFHxS; however, concentrations in serum, liver, urine and feces are reported as PFHxS anion, and percent recoveries of administered dose in those matrices are corrected for the potassium salt. Potassium perfluorooctanoate (K^+PFOA, >99% purity), used as an internal standard for analytical extractions in the IV pharmacokinetic study in cynomolgus monkeys, was supplied by 3M Company (St. Paul, MN). Stable-isotope-labeled $^{18}O_3$-PFHxS ($CF_3(CF_2)_5S(^{18}O_3^-)$), used as an internal standard for extractions in the rat and mouse studies, was supplied by Research Triangle Institute (Research Triangle Park, NC, USA). All other chemicals used were reagent-grade.

2.2. Laboratory animals and husbandry

Studies were completed in the following chronological order: monkeys, rats, and mice. Male and female Sprague- Dawley (SD) rats and CD-1 mice (8–10 weeks old) were purchased from Charles River Laboratory (Wilmington, MA or Portage, MI). All rats and mice were housed in standard cages. Rat chow (Purina Lab Chow or Teklad Mouse/Rat Chow) and tap water were provided *ad libitum* throughout the study except when fasting was required. Environmental controls for the animal room were set to maintain a temperature of 72 ± 3 °F, humidity of 30–70%, a minimum of 10 exchanges of room air per hour and a 12-h light/dark cycle.

e three male and three female cynomolgus monkeys (*Macaca fascicularis*) designated for use in this study were selected from an in-house colony of monkeys that were housed at Southern Research Institute (Birmingham, AL) prior to use on this study. These monkeys were purchased from Charles Rivers BRF, Inc. (Houston, TX). With the exception of one male, these same monkeys previously were given single IV bolus doses of the potassium salts of perfluorobutanesulfonate (PFBS, 10 mg/kg), perfluorobutyrate (PFBA, 10 mg/kg), perfluorohexanoate (PFHxA, 10 mg/kg), and perfluorooctanoate (PFOA, 10 mg/kg) in earlier studies. The studies with PFBA, PFBS, and PFOA have been previously published [34–36]. Certified, commercial, dry monkey chow #5048 (PMI Feeds, Inc., St. Louis, MO) was fed to the monkeys 2–3 times each day. The diet was supplemented with fresh fruit/treats several times each week. Tap water (Birmingham, Alabama public water supply) was available to the monkeys *ad libitum*. The monkeys were housed in a room that was maintained at a temperature of 68–70.3 °F and a relative humidity of 22.2–65.7%. An automatic timer was set to control the room lights which provided 12-h of light/dark cycle per day.

Studies were performed in facilities accredited by the Association for Assessment and for the Accreditation of Laboratory Animal Care International. All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee. Animal care and procedures followed guidelines as specified the U.S. Department of Health and Human Services Guide for the Care and the Use of Laboratory Animals [37].

2.3. Analytical methods

2.3.1. Monkey IV pharmacokinetic study

For the cynomolgus monkey IV pharmacokinetic study, PFHxS standards in serum and urine were prepared in ranges of 5–20,000 ng/mL and 10–500 ng/mL, respectively. Monkey serum or urine samples (0.5 mL) were fortified with an internal standard, perfluorooctanoate (PFOA), followed by the addition of an ion-pairing reagent (tetrabutylammonium hydrogen sulfate) and carbonate/bicarbonate buffer and extraction with ethyl acetate. The ethyl acetate layer was removed by evaporation, and the resulting residue was reconstituted in mobile phase (95% methanol with 1.5% formic acid, 5% 5 mM ammonium acetate). After filtration through a 0.2 μm syringe, the samples were transferred to auto sampler vials and analyzed by LC–MS/MS using an Applied Biosystems-Sciex model API 3000 mass spectrometer (Applied Biosystems/MDS-Sciex Instrument Corporation, Foster City, CA). For PFHxS, the parent negative ion was monitored at 399 atomic mass units (amu). For the internal standard PFOA, the negative ion was transitioned and monitored from 413 → 369 amu.

2.3.2. Pharmacokinetic studies in rats and mice

For studies with rats and mice using K^+PFHxS, serum and urine samples were used as collected. Commercially purchased newborn calf serum (Invitrogen, Carlsbad, CA, USA) was used as blank matrix for preparation of serum matrix standards. Liver, kidney, urine, and feces collected from naïve rats or mice, as appropriate, were used as blank matrix for preparation of matrix-specific standards. Liver or kidney samples were allowed to thaw, and approximately 0.2 g of liver or kidney was weighed and homogenized with deionized water in a clean polypropylene tube. The ratio between liver or kidney and water was 1:4 (w/w). After the primary homogenization step, the whole homogenate was further sonicated for 30 min. Fecal samples were allowed to thaw and then the entire fecal sample for each rat or mouse was weighed and homogenized with deionized water in a clean polypropylene tube. The ratio between feces and water was 1:3 (w/w). After the primary homogenization step, the whole homogenate was centrifuged at 2500 × *g* for 20 min and the corresponding supernatant was referred to as fecal extract.

To prepare matrix-matched PFHxS standard curves, a PFHxS solution prepared in methanol was aliquoted volumetrically to clean polypropylene tubes followed by the addition of 100 μL of the appropriate blank matrix (serum, urine, liver, kidney, or fecal extract) to each tube. The matrix-matched standard curves for PFHxS ranged from 10 to 1000 ng/mL.

One-hundred (100) μL of the processed samples were aliquoted into clean polypropylene tubes followed by the addition of internal standard, $^{18}O_3$-PFHxS. One mL of 1.0 N formic acid was added to all tubes, followed by 100 μL saturated ammonium sulfate. Samples were vortexed between each addition. All samples were extracted using solid phase extraction (SPE). Details for SPE and LC–MS/MS conditions were same as perfluorooctanesulfonate and they have been described previously [38–40]. Briefly, the SPE method utilized Oasis® HLB cartridges (Waters Corporation, Milford, MA, USA) and used $^{18}O_3$-PFHxS as an internal standard. Applied Biosystems Sciex model API 4000 mass spectrometer (Applied Biosystems/MDS-Sciex Instrument Corporation, Foster City, CA) was used for the analysis. PFHxS ion transitions monitored were 399 → 80 amu for PFHxS anion and 405 → 86 amu for the $^{18}O_3$-PFHxS internal standard.

2.4. Rats

2.4.1. Dose effect on elimination

To determine the effect of increasing dose on the clearance profile of PFHxS in the serum, liver, urine, and feces of male and female Sprague Dawley rats within 96 h following a single oral dose, rats ($N = 4$/sex/group) were given single oral doses of 1, 10, or 100 mg K^+PFHxS/kg body weight. The rats were placed in metabolism caging and urine and feces were collected every 24 h after dosing for 96 h. Clinical observations were made after dosing and periodically until euthanasia by CO_2 asphyxiation at 96 h, at which time gross necropsies were performed. Serum (processed from blood) and liver samples were harvested and stored at −80 °C pending LC–MS/MS analysis as previously described.

2.4.2. IV and oral pharmacokinetics of PFHxS in jugular-cannulated rats after a single dose of K^+PFHxS

Male and female Sprague Dawley jugular-cannulated rats ($N = 3$/sex/dose group) were administered a single dose 10 mg K^+PFHxS/kg body weight either by tail-vein IV injection or by oral gavage. K^+PFHxS solution was prepared in saline (for IV study) or distilled water (for oral gavage). Interim blood samples (approximately ~0.5 mL) were collected from cannula to obtain serum at 0.25, 0.5, 1, 2, 4, 8, 18, and 24 h post-dose. Serum samples stored at −80 °C pending analysis for PFHxS by LC–MS/MS as previously described.

2.4.3. Ten-week study on the elimination of PFHxS in serum, urine, and feces after a single IV dose of K^+PFHxS

Male and female Sprague Dawley rats ($N = 4$/sex) were given a single IV dose of 10 mg K^+PFHxS/kg body weight. The K^+ PFHxS solution was prepared in saline. Periodic interim serum (obtained from tail vein bleeding), urine, and fecal samples were collected throughout the study for 10 weeks post-dose. At study termination,

rats were euthanized via CO_2 asphyxiation, blood (collected via abdominal aorta) and liver samples were harvested. All samples taken, including serum, urine, feces, and liver, were frozen with liquid nitrogen and stored at −80 °C pending analysis for PFHxS by LC–MS/MS as previously described.

2.5. Serum uptake and urinary and fecal elimination in mice

Male and female CD-1 mice were given a single oral dose of either 1 or 20 mg K^+PFHxS/kg body weight. The K^+PFHxS solution was prepared in vehicle (0.5% Tween 20). At designated times (2, 4, and 8 h post-dose and days 1, 8, 15, 22, 36, 50, 64, and 162 post-dose), groups of mice (N=4/sex/dose group) were euthanized via CO_2 asphyxiation. Blood (collected via abdominal aorta and processed to serum), kidneys, and liver samples were harvested. In addition, 24-h urine and feces were collected from mice prior to the day of necropsy. Serum samples were obtained after blood clotting and centrifugation (2000 × g, 15 min). All samples taken, including serum, urine, feces, kidneys, and liver, were frozen with liquid nitrogen and stored at −80 °C pending analysis for PFHxS by LC–MS/MS.

2.6. IV pharmacokinetic study in cynomolgus monkeys

K^+PFHxS was dissolved in sterile saline, USP (Phoenix Pharmaceutical Company, St. Joseph, MO; Lot 8101050) at 5 mg/mL. The formulation was stored refrigerated and used for dosing within 1 day after preparation; it was considered to be stable during this period. Dosing solution concentration and homogeneity analyses were not performed.

On day 0, each of the three male and three female cynomolgus monkeys received a single IV dose of K^+PFHxS at 10 mg/kg into a superficial arm or leg vein. Doses were administered at a volume of 2 mL/kg based on the day 0 body weights. All monkeys were observed twice daily for clinical signs. Each monkey was weighed on days 0, 4, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 98, 105, 112, and 119. Urine was collected in standard metabolism cages for 24-h intervals on the following days: prior to dose administration (day −3; baseline), on day 1 (0–24 h post-dose), on day 2 (24–48 h post-dose), and on days 7, 14, 21, 28, 42, 56, and 70. The volume of each urine sample was measured. Urine samples were stored frozen (approximately −20 °C). Fecal samples were also collected but were not analyzed. Blood samples (2 mL) were collected at time 0 (pre-dose); 0.5, 2, 4, 8, 24, and 48 h; and subsequently on days 4, 7, 14, 21, 28, 42, 56, 70, and 171 post-dose. Samples were collected into tubes without anticoagulant and were allowed to clot at room temperature. The blood samples were then centrifuged, and the serum separated and stored at −20 °C until analyzed.

2.7. Data analysis

Selected pharmacokinetic parameters were calculated from the serum PFHxS concentration versus time data using WinNonlin® software (Pharsight Corp.; Mountain View, CA). Data were fit to either a two-compartmental or a non-compartmental model. Statistically significant (p < 0.05) differences in the sex-specific arithmetic means for each pharmacokinetic parameter were determined by the Student's t-test when applicable. Mean values and standard errors (SE) for each parameter were calculated, except in the case of the mouse study, in which the mean by sex for each group of mice at each time point was used. When available, data for the fecal and/or urinary excretion of PFHxS were expressed as a percent of the administered dose eliminated during the collection period. Liver PFHxS concentrations, when available, were expressed as the mean ± SE concentration by sex and group. Statistically significant (p < 0.05) differences in the sex-specific arithmetic means for each pharmacokinetic parameter were determined by the Student's t-test.

3. Results

3.1. Rats

3.1.1. Dose effect on elimination

Results of the experiment to investigate dose effects on elimination are presented in Figs. 1–4. The mean ± standard error (SE) percents of the PFHxS administered dose recovered in serum, liver, urine, and feces 96 h after a single oral dose of either 1, 10, or 100 mg K^+PFHxS/kg body weight as well as the total recovered in those four matrices and the unaccounted percent of dose are presented in Figs. 1 and 2 for males and females, respectively. Regardless of sex, mean serum PFHxS concentrations were non-linear in proportion to dose after 96 h (Fig. 3), and liver concentrations were significantly lower than corresponding serum concentrations. Female serum and liver concentrations were considerably lower than those of males given equivalent doses. This suggested faster serum elimination in female rats, which was also reflected as higher percents of given dose recovered in urine in female rats than in male rats. Urine

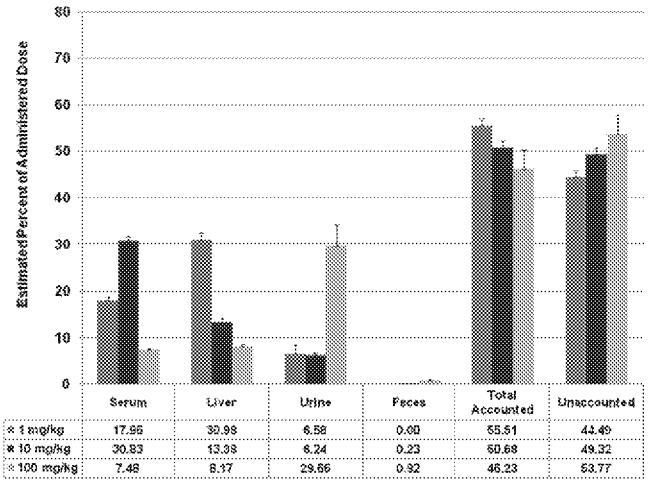

Fig. 1. Mean estimated percent of administered (PFHxS) dose recovered in serum, liver, urine, and feces of male Sprague Dawley rats given a single oral dose of either 1 mg/kg (blue bars), 10 mg/kg (red bars), or 100 mg/kg (green bars) of K^+PFHxS with N=4 rats per dose group. Serum and liver samples were harvested at 96 h post-dose while urine and feces were collected continuously for 96 h post-dose. The total percent accounted and unaccounted for are also illustrated in the chart. Error bars represent standard error. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

was the major route of excretion in male and female rats. Within 96 h following a single oral dose at 1, 10, and 100 mg K^+PFHxS/kg body weight, females excreted 35%, 28%, and 41% of the dose in urine, respectively (Fig. 4). Males excreted only about 6–7% of the dose in urine at the 1 and 10 mg/kg dose level, but excreted 30% at the 100 mg/kg dose level, suggesting that urinary excretion in males is dose-dependent (Fig. 4). In observing daily urinary excretion over the 96-h period following dosing, first-day excretion was significantly higher in both males and females at the 100 mg/kg dose (Fig. 4). Mean daily fecal excretion was <0.5% of administered dose at all time points (data not shown).

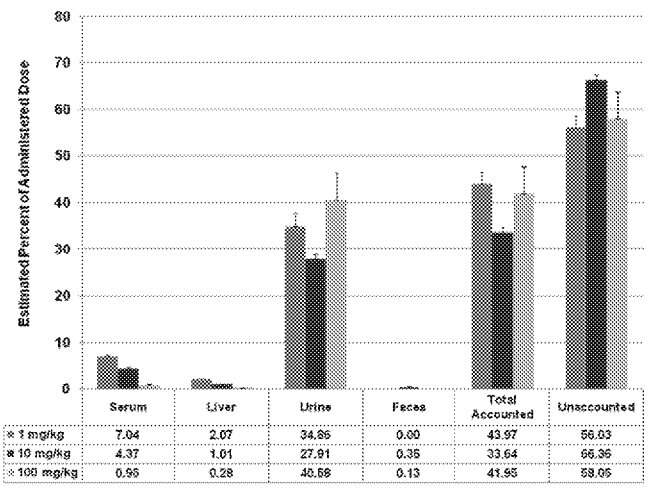

Fig. 2. Mean estimated percent of administered (PFHxS) dose recovered in serum, liver, urine, and feces of female Sprague Dawley rats given a single oral dose of either 1 mg/kg (blue bars), 10 mg/kg (red bars), or 100 mg/kg (green bars) of K^+PFHxS with N=4 rats per dose group. Serum and liver samples were harvested at 96 h post-dose while urine and feces were collected continuously for 96 h post-dose. The total percent accounted and unaccounted for are also illustrated in the chart. Error bars represent standard error. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

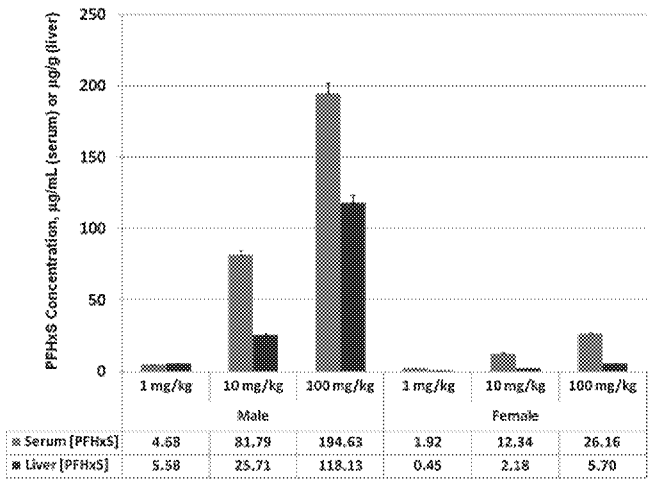

Fig. 3. Mean PFHxS concentration in serum (μg/mL) and liver (μg/g) of male and female Sprague Dawley rats ($N = 4$/sex/dose group) at 96 h post-dose following a single oral dose of either 1, 10, or 100 mg K^+PFHxS/kg body weight. Blue bars represent serum, and red bars represent liver. Error bars represent standard error. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

3.1.2. IV and oral pharmacokinetics of PFHxS in jugular-cannulated rats after a single dose of K^+PFHxS

Presented in Table 1 are the pharmacokinetic parameters calculated from serum concentrations of PFHxS obtained from jugular-cannulated rats that received either a single IV or a single oral dose of 10 mg K^+PFHxS/kg body weight. A two-compartmental model best fit the data.

For the IV study, although three male rats were dosed, due to lack of significant elimination over the 24-h period, serum values from two male rats did not fit the model, hence it was not possible to calculate parameters reliably for males. For females, mean pharmacokinetic values (expressed as mean ± SE) for C_{max}, $T_{1/2}$ α, $T_{1/2}$ β, clearance (CL), volume of distribution at steady state

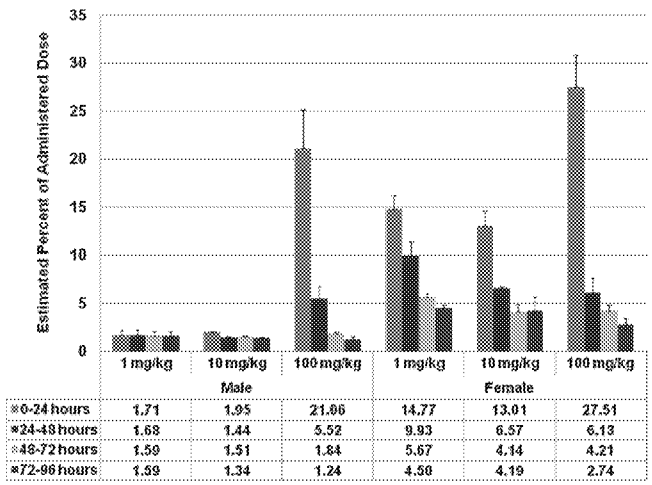

Fig. 4. Mean estimated percent of administered (PFHxS) dose recovered in urine of male and female Sprague Dawley rats given a single oral dose of either 1, 10, or 100 mg K^+PFHxS/kg body weight ($N = 4$/sex/dose group) and 24-h urine samples were collected for 96 h post-dose. Blue bars represent urine samples collected between 0 and 24 h post-dose, red bars represent urine samples collected between 24 and 48 h post-dose, green bars represent urine samples collected between 48 and 72 h post-dose, and violet bars represent urine samples collected between 72 and 96 h post-dose. Error bars represent standard error. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Mean ± SE estimated values for pharmacokinetic parameters in jugular-cannulated Sprague Dawley rats ($N = 3$/sex) given either a single oral or a single IV dose of 10 mg K^+PFHxS/kg body weight and followed up for 24 h based on a two-compartment model.

Parameter	Sex	Oral	IV
T_{max} (day)	Male	N/A[a]	N/A
	Female	0.02 ± 0.01	N/A
C_{max} (μg/mL)	Male	N/A	60.40[b]
	Female	63.57 ± 3.39	58.70 ± 9.56
Serum $[PFHXS]_{last}$ (μg/mL)	Male	61.30 ± 3.48	31.47 ± 8.72
	Female	29.82 ± 1.68	27.15 ± 6.40
λ_α (1/day)	Male	N/A	90.72[b]
	Female	57.72 ± 11.16[c]	41.28 ± 10.23
λ_β (1/day)	Male	N/A	0.096[b]
	Female	0.300 ± 0.228[c]	0.400 ± 0.080
$T_{1/2}$ α (day)	Male	N/A	0.008[b]
	Female	0.073 ± 0.003[c]	0.020 ± 0.006
$T_{1/2}$ β (day)	Male	N/A	6.83[b]
	Female	0.83 ± 0.53[c]	1.83 ± 0.26
CL (mL/day/kg)	Male	N/A	40.32[b]
	Female	N/A	119 ± 47
AUC (μg day/mL)	Male	N/A	248[b]
	Female	54.94 ± 45.31[c]	109.35 ± 31.55
Vd_{ss} (mL/kg)	Male	N/A	N/A
	Female	N/A	278 ± 66

[a] Not available, could not be estimated.
[b] Parameters estimated was based on $N = 1$ rat.
[c] Parameters estimated was based on $N = 2$ rats.

(Vd_{ss}) were 58.70 ± 9.56 μg/mL, 0.020 ± 0.006 day, 1.83 ± 0.26 day, 119 ± 47 mL/day/kg, and 278 ± 66 mL/kg, respectively.

For the oral gavage study, again, it was not possible to estimate parameters reliably for males due to lack of significant serum elimination over the 24-h period. In addition, although three female rats were dosed, serum values from one female rat did not fit the model due to lack of significant elimination over the 24-h period, hence pharmacokinetic parameter estimation was obtained based on two female rats. For females, mean pharmacokinetic values (expressed as mean ± SE) for T_{max}, C_{max}, $T_{1/2}$ α, $T_{1/2}$ β were 0.02 ± 0.01 day, 63.57 ± 3.39 μg/mL, 0.073 ± 0.003 day, and 0.83 ± 0.53 day. CL and Vd_{ss} could not be estimated.

Based on the IV study, the mean serum elimination half-lives ($T_{1/2}$) in male and female rats were 6.83 days (based on data from one male rat) and 1.83 ± 0.26 days, respectively. These values likely are not reliable due to the short duration (24 h) of the observation period.

Comparing the AUC values obtained from the IV and oral studies in female rats yields a bioavailability of 50%. Again, due to the short duration of the observation period, this value for bioavailability based on AUC estimates may not be reliable. The female C_{max} values did not differ significantly between the oral and IV doses, and T_{max} after oral dosing was estimated to be at approximately 30 min. These latter observations suggest approximately complete bioavailability.

3.1.3. Ten-week study on the elimination of PFHxS in serum, urine, and feces after a single IV dose of K^+PFHxS in rats

The means ± SE for several pharmacokinetic parameters estimated from rats given a single IV dose of 10 mg K^+PFHxS/kg body weight and observed for 10 weeks are presented in Table 2. Also included in Table 2 are serum and liver PFHxS concentrations upon terminal sacrifice.

After a single IV dose at 10 mg/kg, mean C_{max} serum PFHxS concentrations were lower in males than in females

Table 2
Mean ± SE estimated values for pharmacokinetic parameters in Sprague Dawley rats (N=4/sex) given a single IV dose of K⁺PFHxS at 10 mg/kg and followed up for 10 weeks based on use of a two-compartment model for males and one-compartment model for females.

Parameter	Sex	Value
T_{max} (day)	Male	N/A[a]
	Female	N/A
C_{max} (μg/mL)	Male	61.87 ± 1.90
	Female	79.10 ± 8.58
Serum $[PFHxS]_{last}$ (μg/mL)	Male	6.25 ± 1.06
	Female	<LLOQ[b]
Liver $[PFHxS]_{last}$ (μg/g)	Male	6.62 ± 0.68
	Female	<LLOQ[c]
% PFHxS dose in urine, 0–24 h	Male	0.70 ± 0.07
	Female	13.28 ± 2.88
λ_α (1/day)	Male	0.7428 ± 0.1362
	Female	0.4226 ± 0.0190
λ_β (1/day)	Male	0.0238 ± 0.0005
	Female	N/A
$T_{1/2}$ α (day)	Male	0.96 ± 0.18
	Female	1.64 ± 0.08
$T_{1/2}$ β (day)	Male	29.1 ± 0.6
	Female	N/A
CL (mL/day/kg)	Male	6.71 ± 0.06
	Female	53.35 ± 4.38
AUC (μg day/mL)	Male	1490 ± 12
	Female	187 ± 15
Vd_{ss} (mL/kg)	Male	275 ± 5
	Female	126 ± 14

[a] Not available, could not be estimated.
[b] LLOQ (lower limit of quantification) for serum = 10 ng/mL.
[c] LLOQ (lower limit of quantification) for liver = 50 ng/g.

(61.87 ± 1.90 μg/mL and 79.10 ± 8.58 μg/mL, respectively). At the end of the 10-week follow-up period, mean serum PFHxS concentration in males was 6.25 ± 1.06 μg/mL (Fig. 5). In contrast, the mean serum PFHxS concentration in females was below the lower limit of quantitation (LLOQ, 0.01 μg/mL) from day 16 post-dose through study termination. The serum PFHxS elimination appeared to be bi-phasic in male rats. In the initial elimination phase (α phase), mean λ for serum elimination of PFHxS were different between males and females (0.7428 ± 0.1362 day^{-1} and 0.4226 ± 0.0190 day^{-1}, respectively). In the second phase of elimination (terminal or β phase), mean λ for serum elimination of PFHxS could only be estimated in male rats, and was 0.0238 ± 0.0005 day^{-1}, resulting in an estimated serum $T_{1/2}$ of 29.1 ± 0.6 days. Serum $T_{1/2}$ of PFHxS in female rats was likely a single-phase event, which was estimated to be 1.64 ± 0.08 days based on λ_α.

At the end of the study, mean PFHxS concentration in liver were similar to the paired serum PFHxS concentrations in male rats. However, in females at study termination, mean PFHxS concentration in liver and serum were below LLOQ (50 ng/g and 10 ng/mL, respectively). Mean Vd_{ss} for male and female rats were 275 ± 5 and 126 ± 14 mL/kg, respectively. The estimated percent of administered PFHxS dose excreted during overnight urine and fecal collections are shown in Figs. 6 and 7, respectively. Mean urinary excretion of PFHxS in females was much higher than that in males during the first week of the study. Mean daily fecal excretion never exceeded 0.5% of the administered dose and, for the majority of measurements, was ≤0.03% of administered dose.

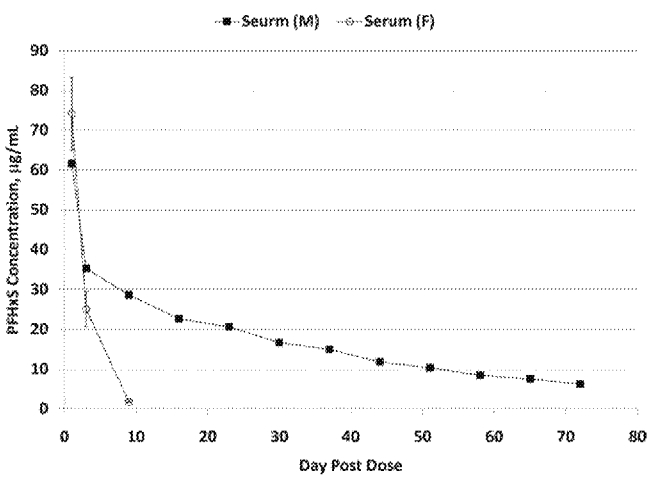

Fig. 5. Mean serum PFHxS concentrations (μg/mL) in male (solid squares) and female (open circles) Sprague Dawley rats (N=4/sex) over time after a single IV dose of 10 mg K⁺PFHxS/kg body weight. Error bars represent standard error.

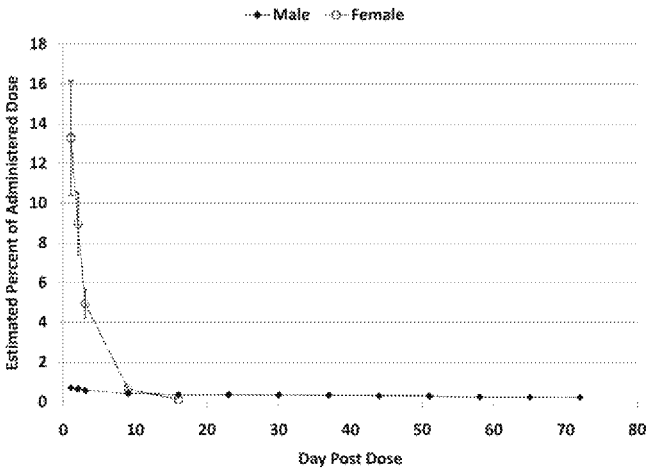

Fig. 6. Mean percent (%) PFHxS dose eliminated in urine in male (solid diamonds) and female (open circlies) Sprague Dawley rats (N=4/sex) at various time after a single IV dose of 10 mg K⁺PFHxS/kg body weight. Each data point represents a 24-h collection period. Error bars represent standard error.

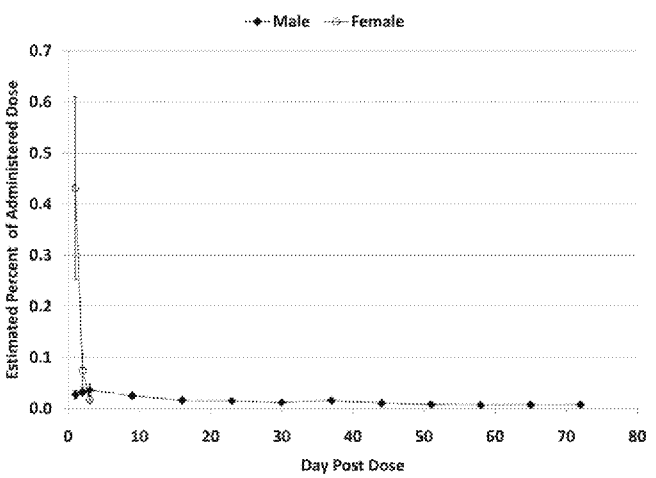

Fig. 7. Mean percent (%) PFHxS dose eliminated in feces in male (solid diamonds) and female (open circlies) Sprague Dawley rats (N=4/sex) at various time after a single IV dose of 10 mg K⁺PFHxS/kg body weight. Each data point represents a 24-h collection period. Error bars represent standard error.

Table 3
Estimated pharmacokinetic parameters in CD-1 mice ($N = 4$/sex/dose group/time point) given a single oral dose of K^+PFHxS at 1 or 20 mg/kg and followed for 23 weeks (162 days) based on a non-compartmental model.

Parameter	Sex	1 mg/kg	20 mg/kg
T_{max} (day)	Male	0.33	0.17
	Female	2	0.17
C_{max} (μg/mL)	Male	8.35	139
	Female	9.85	184
Serum $[PFHxS]_{last}$ (μg/mL)	Male	0.19 ± 0.04	2.06 ± 0.60
	Female	0.12 ± 0.04	1.67 ± 0.55
% PFHxS dosed in urine, 0–24 h		0.882 ± 0.346	1.654 ± 0.511
	Female	0.317 ± 0.137	2.552 ± 1.016
λ (1/day)	Male	0.0227	0.0248
	Female	0.0279	0.0259
$T_{1/2}$ (day)	Male	30.5	27.97
	Female	24.82	26.81
CL (mL/day/kg)	Male	2.94	4.83
	Female	2.68	3.79
AUC (μg day/mL)	Male	340	4138
	Female	372	5270
Vd (mL/kg)	Male	129	195
	Female	96	147

Table 4
Mean ± SE for % dose recovered in 24-h urine and feces in male and female CD-1 mice ($N = 4$/sex/dose group/time point) given a single oral dose of K^+PFHxS at 1 or 20 mg/kg and followed for 23 weeks (162 days).

Time (day)	1 mg K^+PFHxS/kg body weight dose group		20 mg K^+PFHxS/kg body weight dose group	
	Male	Female	Male	Female
PFHxS dose recovered in 24-h urine				
2	0.882 ± 0.346	0.317 ± 0.137	1.654 ± 0.511	2.552 ± 1.016
8	0.815 ± 0.265	1.177 ± 0.184	0.766 ± 0.407	1.726 ± 0.257
15	0.730 ± 0.182	0.599 ± 0.259	0.189 ± 0.287	<LLOQ[a]
22	0.537 ± 0.279	0.684 ± 0.096	0.516 ± 0.303	0.455 ± 0.185
36	0.485 ± 0.059	0.528 ± 0.099	0.513 ± 0.079	0.231 ± 0.145
50	0.217 ± 0.116	0.277 ± 0.028	0.156 ± 0.059	0.140 ± 0.010
64	0.165 ± 0.071	0.135 ± 0.107	<LLOQ	<LLOQ
162	<LLOQ	<LLOQ	0.006 ± 0.006	<0.010 ± 0.005
% PFHxS dose recovered in 24-h feces				
2	1.120 ± 1.398	<LLOQ[b]	0.051 ± 0.024	0.226 ± 0.094
8	<LLOQ	<LLOQ	0.032 ± 0.015	0.139 ± 0.029
15	<LLOQ	<LLOQ	0.038 ± 0.018	0.052 ± 0.019
22	<LLOQ	<LLOQ	0.006 ± 0.005	0.095 ± 0.042
36	<LLOQ	<LLOQ	<LLOQ	0.047 ± 0.011
50	<LLOQ	<LLOQ	<LLOQ	0.023 ± 0.008
64	<LLOQ	<LLOQ	<LLOQ	0.013 ± 0.002
162	<LLOQ	<LLOQ	<LLOQ	<LLOQ

[a] LLOQ (lower limit of quantification) for urine = 10 ng/mL.
[b] LLOQ (lower limit of quantification) for feces = 40 ng/g.

3.2. Mice

Table 3 provides the several pharmacokinetic parameters estimated in male and female mice from data obtained after single oral doses of 1 or 20 mg K^+PFHxS/kg body weight. By dose groups, mean PFHxS concentrations for serum, kidneys, and liver are presented in Fig. 8 (1 mg/kg K^+PFHxS) and Fig. 9 (20 mg/kg K^+PFHxS). Mean PFHxS concentrations were highest in serum followed by liver and then kidney. Regardless of dose, mean serum elimination $T_{1/2}$ values were quite similar between male and female mice (30.50 days versus 24.82 days at 1 mg/kg and 27.97 versus 26.81 days at 20 mg/kg for males and females, respectively). Less than 3% of the administered PFHxS dose was recovered in the urine and feces at any given 24-h sample collection period (Table 4). Urinary elimination predominated. There was no clear indication of a major sex-related difference; although, female mice that received 20 mg/kg dose had a higher percent dose recovered in urine and feces than male mice receiving the same dose during initial collections. Although lower than the Vd values obtained in rats, Vd estimates in mice were still in a range consistent with a predominant extracellular distribution (Table 3).

3.3. Monkeys

Presented in Table 5 are pharmacokinetic parameters in cynomolgus monkeys upon the administration of a single IV dose of 10 mg/kg K^+PFHxS. No adverse clinical signs related to dosing with PFHxS were noted in these monkeys and each monkey either gained weight or maintained weight during the course of

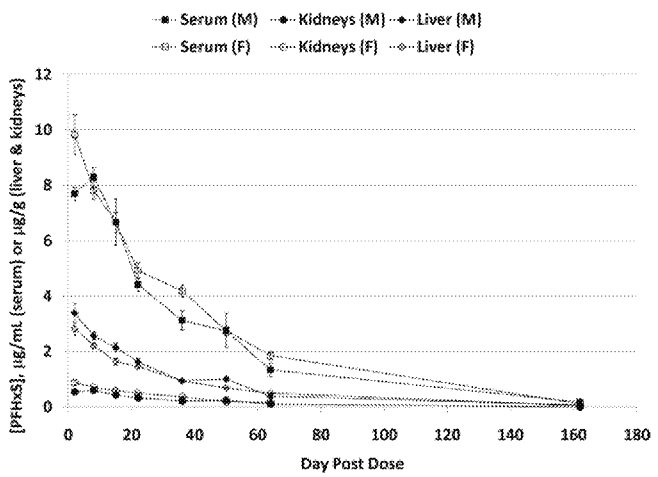

Fig. 8. Mean serum, kidney, and liver PFHxS concentrations in μg/mL (serum) or μg/g (kidney and liver) for male and female CD-1 mice ($N = 4$/sex/time point) over 162 days following a single oral dose of 1 mg K^+PFHxS/kg body weight. Male mouse data for serum, kidney, and liver are illustrated with solid squares, circles, and diamonds, respectively. Female mouse data for serum, kidney, and liver are illustrated with open squares, circles, and diamonds, respectively. Error bars represent standard error.

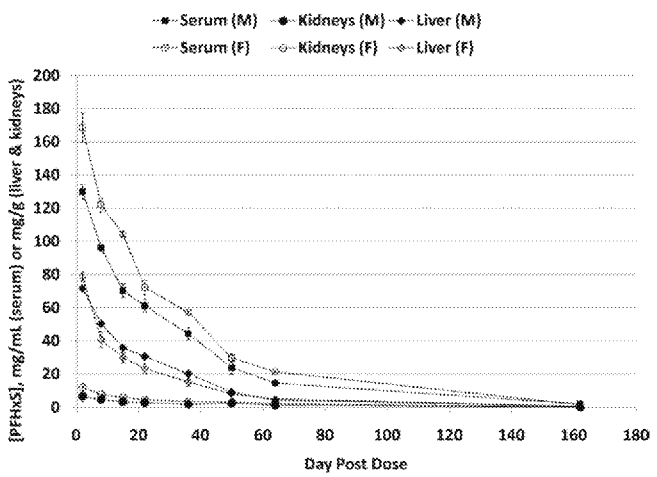

Fig. 9. Mean serum, kidney, and liver PFHxS concentrations in μg/mL (serum) or μg/g (kidney and liver) for male and female CD-1 mice ($N = 4$/sex/time point) over 162 days following a single oral dose of 20 mg K^+PFHxS/kg body weight. Male mouse data for serum, kidney, and liver are illustrated with solid squares, circles, and diamonds, respectively. Female mouse data for serum, kidney, and liver are illustrated with open squares, circles, and diamonds, respectively. Error bars represent standard error.

Table 5
Mean ± SE estimated values for pharmacokinetic parameters in cynomologus monkeys (N = 3/sex) given a single IV dose of K⁺PFHxS at 10 mg/kg and followed up for 171 days based on a non-compartmental model.

Parameter	Male	Female
Serum $[PFHxS]_{0.5\,h}$ (μg/mL)	108 ± 20	148 ± 17
Serum $[PFHxS]_{24\,h}$ (μg/mL)	35.20 ± 5.15	39.50 ± 9.73
Serum $[PFHxS]_{day\ 171}$ (μg/mL)	17.27 ± 2.41	11.73 ± 4.91
% Dose in urine, 0–24 h	0.102 ± 0.010	0.055 ± 0.033
C_{max} (μg/mL)	180 ± 17	180 ± 20
$T_{1/2}$ (day)	141 ± 30	87 ± 27
CL (mL/day/kg)	1.33 ± 0.12	1.93 ± 0.41
AUC (μg day/mL)	7462 ± 675	5794 ± 1396
Vd_{ss} (mL/kg)	287 ± 52	213 ± 28

the study (Fig. 10). Mean serum concentrations at 24 h for males and females were 35.20 ± 5.15 μg/mL and 39.50 ± 9.73 μg/mL, respectively. PFHxS was still quantifiable in serum at day 171 (17.27 ± 2.41 μg/mL and 11.73 ± 4.91 μg/mL in males and females, respectively). For all monkeys, very low levels (~0.03% of the administered PFHxS dose) was consistently recovered in the urine during any given 24-h period of sample collection during the study with no clear indication of a sex-related difference in the urinary excretion of PFHxS. Concentration-versus-time data were best fit to a non-compartmental model (Fig. 11). Mean serum elimination half-lives for PFHxS were less for females than males (87 ± 27 days versus 141 ± 30 days, respectively); however, this difference was not statistically significant (Student's t test, $p > 0.05$) within the limits of study design. Similar to rats and mice, the mean Vd_{ss} suggested predominantly extracellular distribution.

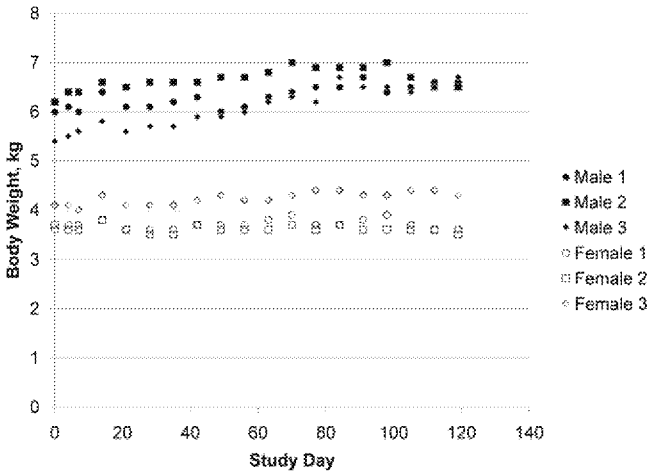

Fig. 10. Individual cynomologus monkey body weight data (kg) over time after a single IV dose of 10 mg K⁺PFHxS/kg body weight. Solid circles, squares, and diamonds represent male monkey weight data while open circles, squares, and diamonds represent female monkey weight data.

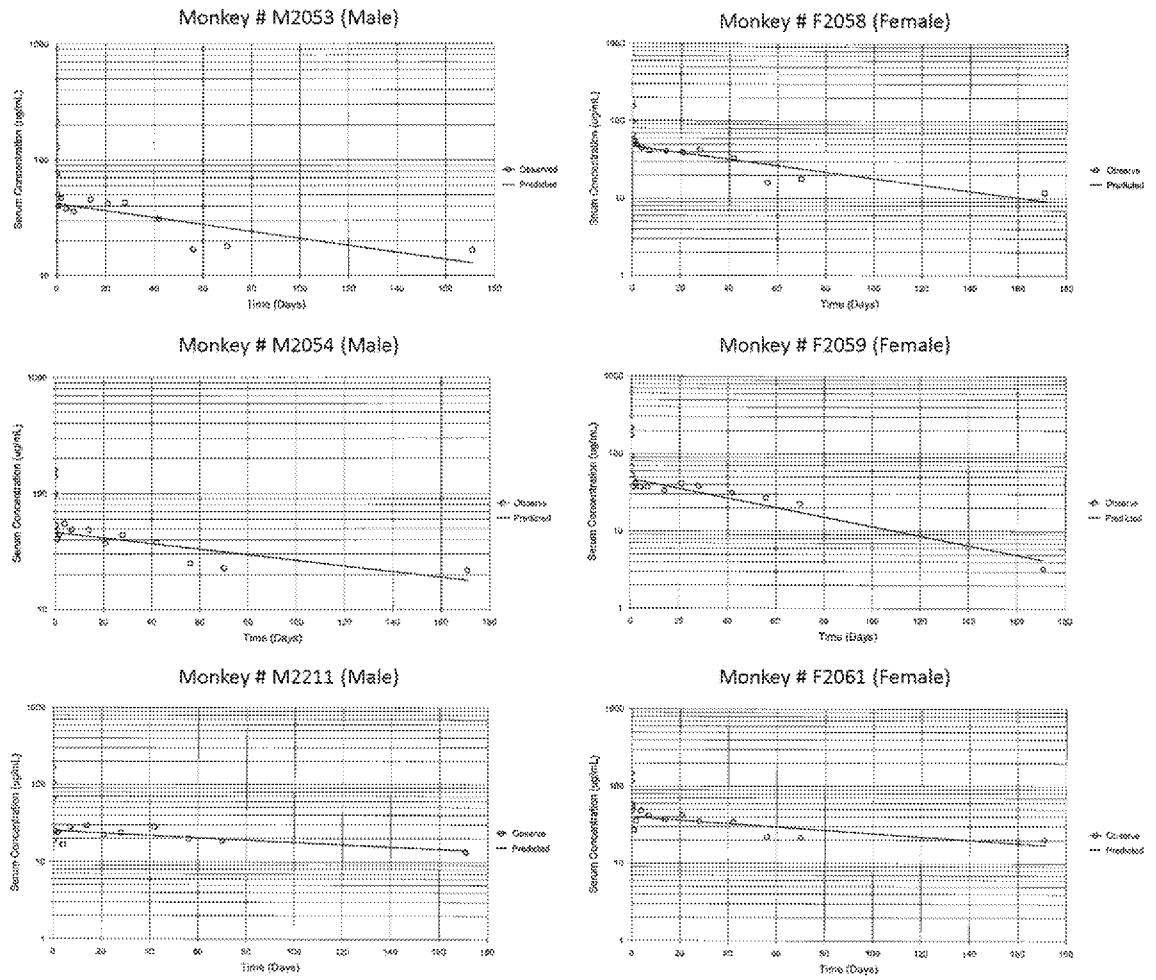

Fig. 11. Serum PFHxS concentrations (μg/mL) in male (left column) and female (right column) cynomologus monkeys (N = 3/sex) over time after a single IV dose of 10 mg K⁺PFHxS/kg body weight. Error bars represent standard error.

4. Discussion

The geometric mean human serum PFHxS elimination half-life has previously been estimated to be approximately 2665 days (95% CI = 2117–3358 days) [5]. To further understand the species differences in elimination, the results from this series of studies have established pharmacokinetic parameters of PFHxS for the rat, mouse, and monkey. The present work provides not only classical pharmacokinetic parameters from short-term studies, but also evaluation of PFHxS elimination over extended time periods. The reported data are of value in interpreting the results of toxicological and epidemiological studies for human health risk assessment.

With the exception of one male monkey, the monkeys used in the present study had also been used previously to study the pharmacokinetics of PFBS, PFBA, PFHxA, and PFOA. These prior studies were conducted over an approximately 10-month period (from April 10, 2000 until February 9, 2001). Based on last drawn serum concentrations below 5 ng/mL and the serum elimination half-lives determined for PFBS [13], PFBA [12], and PFHxA (1.5 and 0.8 days for males and females, respectively, unpublished) and the time elapsed between the last serum sample for these three compounds and the date of dosing for PFHxS on February 9, 2001, no interference would be expected between these three compounds and PFHxS. The last draw for PFOA on February 9, 2001, revealed that the males were at or approaching the method LLOQ (20 ng/mL) and the females averaged approximately 3000 ng/mL serum PFOA concentrations. Because PFHxS was administered on the same day as the last PFOA draw, it is possible that some interaction between PFOA and PFHxS kinetics may have occurred.

The principal differences observed between species in the pharmacokinetic parameters measured were in elimination rates. Rats and mice appeared to be more effective at eliminating PFHxS than monkeys, with female rats demonstrating much more rapid elimination than male rats and male and female mice. With the exception of female rats, which had serum PFHxS elimination half-life of approximately 2 days, the serum elimination half-lives in the rodent species followed over extended time periods (10–23 weeks) were on the order of 1 month; whereas, in monkeys, the serum elimination half-lives approximated 4 months when studied over a 24-week period. Within the first 24 h after K^+PFHxS administration, at least 0.7% or more of the administered PFHxS dose was recovered in the urine in rats and mice. The urinary recovery was between 0.05 and 0.1% in monkeys for the initial 24 h, consistent with the slower elimination observed in cynomolgus monkeys than in rodents. Volumes of distribution at steady state (Vd_{ss}) estimated for the species studied (rats, mice, and monkeys) appeared to be similar. Although Vd_{ss} from female rats was likely an underestimate due to the fast elimination of PFHxS in serum, it would be reasonable to conclude that the Vd_{ss} in the three species studied likely is in the range of 200–300 mL/kg body weight based on review of all the data. Thus, PFHxS appears to be distributed predominantly in extracellular space.

The difference in serum elimination half life between sexes in monkeys and mice were not statistically significant under study conditions. In contrast, large differences between male and female rats were evident. In rats, sex differences in pharmacokinetics have been noted for perfluorobutyrate (PFBA) [34], PFOA (first observed by Griffith and Long [41] and reviewed by Kudo and Kawashima [42]) and PFOS (in this issue) [43]. In the study reported herein for PFHxS, the estimated serum elimination half-life for female rats was less than 2 days based on IV studies; whereas, estimated mean serum elimination half-lives of 6.83 or 29.1 days were obtained for males after IV administration and follow-up periods of 24 h or 10 weeks, respectively. In studying the elimination of linear and branched PFOS in male Sprague Dawley rats, Benskin et al. [44] also followed the elimination of linear and branched PFHxS, a contaminant of their PFOS sample, for 38 days after dosing. The effective oral dose of PFHxS in the Benskin et al. study was 30 μg/kg. They found that branched PFHxS was preferentially eliminated over the linear form, with serum elimination half-lives of 3.5–6.9 days for branched forms and 15.9 days for linear PFHxS. Although linear and branched isomers were not distinguished from one another in the present studies reported herein, the K^+PFHxS sample used contained 3.8% branched isomers. Based on our observations and those of Benskin et al., serum elimination half-life data for PFHxS based on relatively short-term follow-up periods likely overestimates the rate of elimination over a more extended period. This overestimation in shorter-duration studies may be due to an initial, more rapid phase elimination of PFHxS in male rats, as shown by the time-dependent decrease in % PFHxS dose recovered in urine and feces (see Figs. 6 and 7) as well as the potential for early elimination of branched isomers.

The elimination kinetics of PFHxS by species are similar to those of PFOS in mice, male rats, monkeys, and humans. Of the perfluoroalkyls for which pharmacokinetic data are available, including PFBA [34], PFHxA [45], PFOA [5,36,46–51], PFBS [35], and PFOS [5,44,52], PFHxS and PFOS have the lowest elimination rates. Both PFBA and PFHxA appear to be eliminated the most efficiently in the species studied (mice, rats, monkeys, and humans for PFBA, and rats and monkeys for PFHxA), with serum elimination half-lives of hours to several days. PFBS half-lives of elimination were in the range of those reported for PFBA and PFHxA for rats and monkeys (mice have not been studied); however, the estimated human elimination half-life of approximately 1 month contrasts to the estimated 3-day human serum PFBA elimination half-life. Serum PFOA elimination rates in mice and monkeys are similar with half-lives of approximately 2–3 weeks, while male rats half-life is approximately 1 week, the female rat half-life a matter of hours, and, in humans, estimated at 3.5 years (geometric mean, 95% CI = 3.0–4.1 years).

Differential expression of organic anion transporters among species and sex within species, as well as developmental stage, may influence the similarities and differences observed in the kinetic profiles of the various perfluoroalkyls. Based on studies conducted with PFOA, it may be reasonable to speculate that the differences in PFHxS elimination between species and the sex difference in PFHxS elimination within species in rats could be due to differences in expression of organic anion transporters. Numerous studies have demonstrated that difference in the elimination of PFOA in rats is mediated by testosterone [53,54] and the potential role of differential (by sex) expression of renal organic anion transporters in rats in the observed sex difference in renal excretion of PFOA was further established by Kudo et al. [55], which included the possible role of apical expression of the uptake transporter, Oatp1, in renal proximal tubules of male rats in facilitating reabsorption of PFOA from the urinary filtrate. Hinderliter et al. [49] further showed that the enhanced urinary excretion of PFOA in female rats developed concurrently with sexual maturation. Furthermore, PFOA has been demonstrated to be a potential substrate for renal organic anion transporters in rats [56–58] and humans [59,60].

Analysis of the kinetic data from the single-dose IV study in monkeys with K^+PFHxS reported herein as well as with K^+PFOS [43] and K^+PFOA [36] in the same set of male and female cynomolgus monkeys (with the exception of one male) has suggested a potential role for saturable renal tubular reabsorption [61]. Andersen et al. [61] suggested that changing the transport maximum in a human pharmacokinetic model could account for the longer half-life of PFOS in humans as compared to monkeys. At this time, it is not known to what extent the kinetics of the perfluoroalkylsulfonates may be determined by organic anion transporter mediated processes and how these may differ between species and sex within species.

In the only human serum PFHxS elimination study available to date reported by Olsen et al. [5], the geometric mean serum PFHxS elimination half-life among 26 retired fluorochemical workers followed for approximately 5 years was estimated at 2665 days (95% CI = 2117–3358 days). This reported value is in stark contrast to the mean serum PFHxS elimination half-life of 114 days observed among the three male and three female monkeys reported herein as well as the serum PFHxS elimination values reported herein for rats and mice.

Consistent with the low serum PFHxS elimination rate observed in retired workers, Olsen et al. [7] reported an approximately 30% (0.4 ng/mL) decline in geometric mean serum PFHxS among American Red Cross blood donors from six regional blood donation centers in the 5-year period between 1999/2000 and 2006. In the same approximate time period between 1999/2000 and 2005/2006, Kato et al. [11] reported a 22% decrease in the geometric mean United States general population serum PFHxS concentration which was followed in 2007/2008 by a 17% (0.29 ng/mL) increase in the 2005/2006 geometric mean value. However, inspection of their data suggests that serum PFHxS concentrations have been relatively stable with the exception of an apparent decline in the geometric mean in the 2005/2006 sampling period. Whether the decrease in 2005/2006 was real or due to unexplained variability remains unclear. Some insight comes from a longitudinal study of mothers, their children, and men from three cities in Germany [16]. Over a 2-year period from 2006 to 2008 within the same set of individuals, geometric mean serum concentrations of PFHxS were observed to decline by 14–41% in the groups of mothers, their children, and men from the three cities. In reporting on trends in human milk PFHxS concentrations from Stockholm, Sweden from 2001 through 2008, Sundström et al. [17] reported a negative slope of 6.1% per year associated with a halving time of 11 years that was not statistically significant for trend. These biomonitoring studies support the slow elimination of PFHxS from the body or suggest continued exposure, or both.

PFHxS, next to PFOS and PFOA, has been the third most frequently detected perfluoroalkyl in blood-based samples from the general population [6,7,9,10]. The presence of endogenous steroid sulfates in human serum samples has been suggested to cause a possible interference leading to an overestimation of serum PFHxS concentration, because these steroid sulfates share the same MS/MS transition ions (99 or 80 amu) that are monitored routinely during PFHxS analyses [62,63]. However, this issue is a result of the use of methanol as the strong solvent in the mobile phase. Kato et al. [64] have shown that adequate separation of the interfering steroid sulfates was achieved using acetonitrile in place of methanol in the mobile phase. In the studies reported herein, interference by steroid sulfates in serum was obviated by the methods employed. In the monkey study, only parent ion for PFHxS was monitored. In the rat and mouse studies, SPE was used with acetonitrile as the eluting solvent, and the LC–MS/MS analyses used acetonitrile as the strong solvent in the mobile phase. In the methods used for the rat and mouse studies, accuracy and precision were enhanced by the use of the internal standard, $^{18}O_3$-PFHxS, which produced a strong parent ion (405 amu) and the resulting negative product ion $S^{18}O_3^-$ (86 amu) as the quantitative internal standard ion.

In summary, the pharmacokinetic profile of PFHxS was evaluated for multiple species, including rat, mouse, and monkey. There were variations in the serum elimination half-lives of PFHxS across the three species with sex-specific elimination differences observed in the rat but not demonstrated in mice or monkeys. Although these estimates may not represent the same active processes between species, they do allow comparison of serum elimination. As exemplified by these data for PFHxS, differences in pharmacokinetic handling between species, and between sexes within species, underscore the importance of understanding pharmacokinetic handling when estimating exposure and assessing risk.

Conflict of interest statement

Maria Sundström and Åke Bergman are with Stockholm University which received a grant from 3M Company for perfluoroalkyl research. Shu-Ching Chang, David Ehresman, Jill Hart, and John Butenhoff are employees of the 3M Company, a former manufacturer of PFHxS and related materials. Patricia Noker (current active employee) and Gregory Gorman (former employee) of Southern Research Institute were contracted by 3M Company to conduct the IV pharmacokinetic study in monkeys. Major funding for the study was from 3M Company.

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